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Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function.

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What is the central question of this study?

To determine the independent effects of hypoxia and hypocapnia on cerebral haemodynamics and cognitive function.

What is the main finding and its importance?

Our data indicates that exposure to hyperventilation-induced hypocapnia causes cognitive impairment in both normoxia and hypoxia. In addition, supplementation of carbon dioxide during hypoxia alleviates the cognitive impairment and reverses hypocapnia-induced vasoconstriction of the cerebrovasculature. These data provide new evidence for the independent effect of hypocapnia on the cognitive impairment associated with hypoxia.

Abstract

Hypoxia, which is accompanied by hypocapnia at altitude, is associated with cognitive impairment. This study examined the independent effects of hypoxia and hypocapnia on cognitive function and assessed how changes in cerebral haemodynamics may underpin cognitive performance outcomes. Single reaction time (SRT), five-choice reaction time (CRT) and spatial working memory (SWM) tasks were completed in 20 participants at rest and after one hour of isocapnic hypoxia (IH, end-tidal oxygen partial pressure ($P_{ET}O_2$) = 45mmHg, end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) clamped at normal), and poikilocapnic hypoxia (PH, $P_{ET}O_2$ = 45mmHg, $P_{ET}CO_2$ not clamped). A subgroup of 10 participants were also exposed to euoxic hypocapnia (EH, $P_{ET}O_2$ = 100mmHg, $P_{ET}CO_2$ clamped 8mmHg below normal). Middle cerebral artery velocity (MCAv) and prefrontal cerebral haemodynamics were measured with transcranial Doppler and near infrared spectroscopy, respectively. IH did not affect SRT and CRT performance from rest (566 ± 50ms and 594 ± 70ms), whereas PH (721 ± 51ms and 765 ± 48ms) and EH (718 ± 55ms and 755 ± 34ms) slowed response times ($p<0.001$ vs IH). Performance on the SWM task was not altered by condition. MCAv increased during IH compared to PH ($p<0.05$), which was unchanged from rest. EH caused a significant fall in MCAv and prefrontal cerebral oxygenation ($p<0.05$ vs baseline). MCAv was moderately correlated to cognitive performance (R^2=0.266–0.289), whereas prefrontal cerebral tissue perfusion and saturation were not ($p>0.05$). These findings reveal a role of hyperventilation-induced hypocapnia *per se* on the development of cognitive impairment during normoxic *and* hypoxic exposures.

Table of Abbreviations

CANTAB	Cambridge Neuropsychological Test Automated Battery
CaO_2	arterial oxygen content
CBF	cerebral blood flow
$CMRO_2$	cerebral metabolic rate of oxygen
CRT	five-choice reaction time task
EH	euoxic hypocapnia
HCO_3^-	bicarbonate ion
IE	isocapnic euoxic
IH	isocapnic hypoxia
MAP	mean arterial pressure
MCAv	middle cerebral artery velocity
NIRS	near infrared spectroscopy
nTHI	total haemoglobin normalised to the initial value
$PaCO_2$	partial pressure of arterial carbon dioxide
PaO_2	partial pressure of arterial oxygen
$P_{ET}CO_2$	end-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	end-tidal partial pressure of oxygen
PH	poikilocapnic hypoxia
SRT	single reaction time task
SWM	spatial working memory
TCD	transcranial Doppler
TOI	total oxygenation index

Introduction

Exposure to high altitude can cause a number of hypoxia-induced physiological complications such as acute mountain sickness, pulmonary and/or cerebral oedema, and impairment of cognitive function (Hackett & Roach, 2001). Individuals become quickly aware of physical symptoms such as dizziness, headaches and nausea at altitude (Hackett & Roach, 2001), but they are less aware of the impairment to their cognitive function (Asmaro, Mayall, & Ferguson, 2013). The degree to which cognitive function is impaired is related to the severity of the hypoxic stimulus, particularly for tasks that require a higher order of cognitive ability (Petrassi, Hodkinson, Walters, & Gaydos, 2012; Yan, 2014). This higher order ability is essential for decision-making and attentional processes in individuals who venture to unfamiliar and dangerous environments, such as is typical of the high-altitude environment.

The brain relies on two variables to maintain sufficient oxygen supply and its functional capacity; namely, arterial oxygen content (CaO_2) and cerebral blood flow (CBF). During exposure to hypoxia, partial pressure of arterial oxygen (PaO_2) will fall (and related CaO_2) and subsequently the cerebrovasculature dilates in order to increase CBF to maintain global oxygen delivery to the brain (Kety & Schmidt, 1948; Willie, Tzeng, Fisher, & Ainslie, 2014). Simultaneously, the peripheral chemoreceptors activate the hypoxic ventilatory response to increase oxygen intake via the lungs. Consequently, this increased respiration gives rise to hypocapnia, a known vasoconstrictor of the cerebrovasculature (Kety & Schmidt, 1946). Therefore, the change in CBF is influenced by two conflicting stimuli, with the balance of these changes in oxygen and carbon dioxide tensions key factors in the overall change in CBF during exposure to hypoxia (Lucas et al., 2011; Bruce et al., 2016). Given this, hypocapnic-induced vasoconstriction could play a defining role in the cognitive impairment experienced at altitude through compromising cerebral tissue perfusion via its effect on the capacity of the vasculature to dilate in response to hypoxaemia.

To investigate the physiological effects of hypocapnia participants are often instructed to voluntarily hyperventilate. Studies using this method have demonstrated that hypocapnia compromises brain function through its effect on the cerebrovasculature and produces similar impairment to that experienced at altitude, as evidenced by reports of light-headedness and dizziness (Bresseleers, Van Diest, De Peuter, Verhamme, & Van den Bergh, 2010), and impairment of complex cognitive tasks such as Stroop Test performance (Van Diest, Stegen, Van de Woestijne, Schippers, & Van den Bergh, 2000). The ambient gas compositions experienced at altitude are as consequence of a reduction in atmospheric pressure (hypobaric hypoxia), but can be mimicked in the laboratory setting through a reduction in partial pressure of oxygen (normobaric hypoxia). Despite some evidence suggesting different physiological responses between hypobaric hypoxia and normobaric hypoxia (Savourey, Launay, Besnard, Guinet, & Travers, 2003), the ability to tightly control gas composition in the laboratory setting enables the comparison of poikilocapnic hypoxia (PH), as it occurs naturally from hypoxia-induced hyperventilation, to that of isocapnic hypoxia (IH), where the effects of hypoxia *per se* can be separated from hypocapnia by clamping partial pressure of arterial carbon dioxide ($PaCO_2$) at its normal value. Using such an approach, Van Dorp et al. (2007) compared the effects of PH with that of IH on a combination of vigilance and multi-attribute cognitive tasks and found that carbon dioxide supplementation during hypoxia (IH) alleviated the impairment in cognitive function such that performance was similar to that under normoxic conditions. The authors concluded that the hypocapnic element of PH may be directly related to the compromised cognitive function.

However, the independent contribution of hypocapnia to cognitive function and its link to CBF during hypoxia remains unclear. To our knowledge, no study has attempted to separate the roles of hypoxia *and* hypocapnia on cognitive function, as well as the associated changes in cerebral haemodynamics and task performance. Therefore, the present study was designed to examine the isolated effects of hypocapnia and hypoxia on simple and complex cognitive tasks, as well as to explore how changes in global and prefrontal cerebral haemodynamics might relate to changes in cognitive performance.

Methods

Ethical Approval

Ethical approval for this study was provided by the Safety and Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham (reference: MW 07/10/14) and was conducted in accordance with the standards of the *Declaration of Helsinki,* except for registration in a database, with written informed consent obtained from participants before they took part in the study.

Participants

Twenty young healthy males (aged 22.4 ± 6.3 years) participated in this study. All participants completed a general health questionnaire and were invited to participate if they were healthy, non-smokers, and had no history of cardiorespiratory disease. Participants were asked to refrain from consuming alcohol and from undertaking strenuous exercise within 24 hours of each experimental session. Participants were also asked not to consume caffeinated drinks within six hours, and food within two hours prior to reporting to the laboratory.

Study Design and Procedures

All participants visited the laboratory on three occasions, once for a familiarisation session and then for two experimental sessions performed in a random order and separated by at least 48 hours. A subgroup of 10 participants completed a third experimental session. All participants completed an IH session (end-tidal partial pressure of oxygen ($P_{ET}O_2$) = 45mmHg and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) clamped at each participant's normal value) and a PH session ($P_{ET}O_2$ = 45mmHg and $P_{ET}CO_2$ not controlled), while the subgroup completed an additional euoxic hypocapnia (EH) session ($P_{ET}O_2$ = 100 mmHg and $P_{ET}CO_2$ clamped at 8 mmHg below each participant's normal value) (see Figure 2). Participants were blinded to IH and PH conditions only, as participants were coached to maintain a ventilation rate during EH.

Familiarisation

Participants visited the laboratory to familiarise themselves with the equipment and procedures that were used in the study. During this session, participants completed one repeat of the reaction time tasks and three repeats of the spatial working memory (SWM) task of the Cambridge Neuropsychological Test Automated Battery (CANTAB) programme to minimise any learning effect on performance outcomes during the experimental conditions.

Isocapnic Hypoxia (IH)

Participants were comfortably seated while being instrumented to measure cerebral haemodynamics, peripheral arterial oxygen saturation, mean arterial blood pressure and heart rate. The pulse oximeter probe and blood pressure finger cuff were attached to fingers on their non-dominant hand, allowing their dominant hand to be used for the cognitive function tests. Once instrumentation was complete and the signals were optimised, participants breathed through a mouthpiece whilst wearing a nose clip. Control of end-tidal gases was achieved by means of a dynamic end-tidal forcing system described in detail elsewhere (Robbins, Swanson, & Howson, 1982). Participants completed the first battery of cognitive function tests under isocapnic euoxic (IE) conditions ($P_{ET}O_2$ = 100 mmHg and $P_{ET}CO_2$ clamped at participant's normal value). This was followed by a 60-minute intervention period during which participants were exposed to IH, followed by a repeat of the cognitive function tests whilst remaining under IH conditions. Once the cognitive function tests were completed participants were returned to breathing room air and equipment was removed.

Poikilocapnic Hypoxia (PH)

This protocol was identical to the one described for IH except that $P_{ET}CO_2$ was not controlled during the 60-minute intervention and during the repeat of the cognitive function tests.

Euoxic Hypocapnia (EH, n=10)

This protocol was identical to the one described for IH except that participants were exposed to EH during the 60-minute intervention and during the repeat of the cognitive function tests. Hypocapnia was achieved through voluntary hyperventilation. For this, participants were coached to hyperventilate enough to reduce their $P_{ET}CO_2$ to approximately 10 mmHg below their normal value, allowing the dynamic end-tidal forcing system to then adjust $P_{ET}CO_2$ to 8 mmHg below accurately. Figure 1 shows a schematic of the protocol during each experimental visit, as well as examples of each of the CANTAB tests completed under each condition.

Equipment and Measures

Cognitive Function Assessment

Cognitive function was measured via a touch screen CANTAB cognition computer test (Cambridge Cognition Ltd., United Kingdom). The CANTAB is a valid neuropsychological testing instrument of cognitive function (Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013), and is regularly used to assess cognitive function in both healthy and neurodegenerative cohorts (e.g. mild cognitive impairment (Saunders & Summers, 2010) and Alzheimer's disease (Matos Goncalves, Pinho, & Simoes, 2018)). Reaction time tasks and the SWM tests were performed representing simple and complex cognitive tasks respectively.

Reaction Time Tasks

Reaction time was measured through two tasks; single reaction time task (SRT) and five-choice reaction time task (CRT). Both tasks required participants to hold down a pressure pad placed in front of the computer and to tap a circle on the monitor as quickly as possible after a yellow spot was displayed within it. The time taken for the yellow spot to appear was randomised between trials. This task was completed with a single response circle for the SRT, whilst the spot had the option to flash in any one of five response circles in the CRT (see Figure 1a). Participants were given practice attempts of both tasks prior to the test period in which their performance was recorded. Performance time was recorded as the sum of reaction time (time taken between the yellow spot appearing and releasing the pad) and movement time (time taken between releasing the pad and tapping the circle). Additionally, error count (releasing the pad too early or missing the correct circle) was measured for both reaction time tasks.

Spatial Working Memory Task

SWM was measured through a visuospatial task. The participant was presented with a selection of coloured boxes on the screen and the aim was to find all of the tokens hidden inside these boxes. Participants were required to use working memory and a process of elimination to find all of the tokens as only one token was hidden at a time and would never be found in the same box again. Three sets of practice trials (three boxes within each set) were completed before performance was recorded across three stages of increasing difficulty, with each stage consisting of four sets of trials with four, six and eight boxes, respectively, for each level of increasing difficulty. The total number of errors were recorded as the measure of performance. Errors were recorded when participants returned to a box where a token had already been found, or when a box that had been previously selected was selected again in a subsequent search.

Cerebral blood flow velocity and prefrontal cerebral haemodynamics

Bilateral measures of blood flow velocity from the left and right middle cerebral artery (MCAv) were measured using a 2 MHz pulsed Transcranial Doppler (TCD) ultrasound system (Doppler Box, DWL, Compumedics Ltd, Germany) using standardised procedures (Willie et al., 2011). Probes were placed over the left and right temporal windows and secured in place via an adjustable head piece. Photographs of the probe position and angle were used to replicate the placement between sessions, and signal depth and gain settings were also replicated. Left and right side MCAv measures were averaged, reported as a pooled mean, and expressed as a change from resting baseline.

In the subgroup of 10 participants that completed all three protocols prefrontal cerebral haemodynamics was also monitored non-invasively on the left and right side of the forehead using near infrared spectroscopy (NIRS; NIRO-200NX, Hamamatsu Photonics KK; Hamamatsu, Japan). The NIRS probes were housed in light-shielding cases and attached to the forehead skin with tape in the same position for each session. Probes were placed as lateral and superior as possible to avoid the frontal sinus and to allow the TCD head piece to fit between the probes and the superior orbital ridge (i.e. probe centre points were located approximately 4 cm from the midline and approximately 3 cm above the orbital ridge). The NIRO-200NX device measures changes in chromophore concentrations of oxyhaemoglobin and deoxyhaemoglobin via the modified Beer-Lambert law and provides depth-resolved measures of tissue oxygen saturation [total oxygenation index (TOI)] and tissue haemoglobin content (i.e., relative value of the total haemoglobin normalised to the initial value, nTHI) using the spatially resolved spectroscopy (SRS) method. The SRS-derived NIRS parameters limit contamination from superficial tissue via depth-resolved algorithmic methods, providing an index of targeted local tissue saturation (TOI) and perfusion (nTHI) (Davies et al., 2015). Given the inter-individual variability of baseline measures using this imaging technology (Davies et al., 2017) and in accordance with recommendations of others (Subudhi, Miramon, Granger, & Roach, 2009), these NIRS data are expressed as the magnitude of the change from the resting baseline value.

Cerebrovascular haemodynamics, cardiovascular and respiratory variables were all acquired continuously at 200 Hz using an analogue-to-digital converter (Powerlab/16SP ML795; ADInstruments, New Zealand) interfaced and displayed in real time using LabChart software (Chart v7.5, ADInstruments) on a computer.

Data Analysis

Mean SRT and CRT performance time and error count, and SWM task mean error count were collected from each CANTAB trial. A 60 s mean for MCAv, TOI and nTHI data were collected from the two baseline measures that preceded each CANTAB battery under IE or experimental conditions. During CANTAB battery periods, MCAv, TOI and nTHI data were averaged from the final 20 s of each reaction time task (SRT and CRT) and the final 30 s of the SWM task. One participant's TOI data was lost due to corruption of the containing file.

A repeated-measures analysis of variance (IBM SPSS Statistics v23) was used to assess the relations between condition (IH, PH, EH), time (IE, Experimental) and task phase (Baseline, SRT, CRT, SWM) for each physiological variable. A repeated measures analysis of variance was also used to assess the relations between condition (IH, PH, EH) and time (IE, Experimental) for each CANTAB performance variable. Pairwise comparisons (Bonferroni adjusted) were applied to evaluate main effects and interactions. The relationship between changes in selected physiological variables (MCAv, TOI, nTHI) and change in reaction time task performance (SRT and CRT) were determined using Pearson's correlations. Data are presented as mean ± SD and statistical significance was accepted at $p < 0.05$.

Results

There were marked differences between IE baseline and experimental measures of $P_{ET}O_2$, $P_{ET}CO_2$, MCAv, TOI and nTHI (see Table 1). This general pattern was consistent during cognitive testing (see Figures 2 and 3), with no significant differences between the measured time points within each condition (all $p > 0.05$). Nevertheless, we have presented the haemodynamics for each specific time point in Figure 3, but for brevity we have summarised our findings using pooled data across the cognitive tasks and report differences between condition (IH, PH, EH) and time (IE, Experimental) for each dependent variable.

End Tidal Gas Control

Baseline and experimental end-tidal values are shown in Table 1, and a representative example of the differences shown in Figure 2. By design, end-tidal gases were similar during IE conditions, and were successfully manipulated and held consistent during cognitive testing under experimental conditions. Specifically, $P_{ET}CO_2$ remained clamped at IE values during IH (41.1 ± 2.0 mmHg), whereas $P_{ET}CO_2$ declined during the PH (37.4 ± 2.7 mmHg; $p < 0.001$ vs IE and $p < 0.001$ vs IH). For the subgroup completing the EH condition, $P_{ET}CO_2$ was lowered to 32.6 ± 2.3 mmHg ($p < 0.001$ vs IE), significantly lower than IH (40.9 ± 1.8 mmHg; $p < 0.001$) and PH (36.6 ± 3.0 mmHg; $p < 0.01$). The reductions in $P_{ET}O_2$ during IH (44.2 ± 1.7 mmHg) and PH (43.2 ± 2.4 mmHg) interventions were similar (both $p < 0.05$ vs IE). For the subgroup, $P_{ET}O_2$ during the EH condition remained clamped at IE levels (98.6 ± 6.0 mmHg), which was significantly greater than IH (44.2 ± 2.2 mmHg; $p < 0.001$) and PH (43.5 ± 3.2 mmHg; $p < 0.001$).

Haemodynamic Measurements (Isocapnic Euoxic vs Experimental conditions)

Baseline absolute measures of heart rate, mean arterial pressure (MAP), MCAv, nTHI and TOI in IE conditions were consistent between all sessions and are shown in Table 1. There was no difference in heart rate between IE and experimental conditions, whereas there was a main effect of time for MAP ($p < 0.05$) representing elevated values during the experimental conditions compared to IE baseline. Compared to IE, MCAv increased during IH (up 6.7 ± 7.2 $\mathrm{cm \cdot s^{-1}}$; $p < 0.001$ vs IE) whereas it remained similar during PH ($p = 0.63$ vs IE) and thus lower than IH ($p < 0.001$). In the subgroup, similar results for IH (up 6.6 ± 8.5 $\mathrm{cm \cdot s^{-1}}$; $p < 0.05$ vs IE) and PH ($p = 0.16$ vs IE, and $p < 0.05$ vs IH) conditions were seen, while MCAv decreased by 9.2 ± 6.4 $\mathrm{cm \cdot s^{-1}}$ from IE ($p < 0.001$) during the EH condition ($p < 0.01$ vs IH, and $p = 0.18$ vs PH). Measures of prefrontal cerebral haemodynamics collected via NIRS in the subgroup completing all three conditions demonstrated that prefrontal perfusion (as indexed by nTHI) increased from IE for IH (up 0.05 ± 0.05 au; $p < 0.05$ vs IE) and PH (up 0.05 ± 0.08 au; $p = 0.071$ vs IE), while nTHI decreased in EH (down 0.05 ± 0.04 au; $p < 0.05$ vs IE, and $p < 0.05$ IH vs PH). All conditions recorded a significant decline in prefrontal tissue saturation (indexed by TOI) compared to IE ($p < 0.001$), with a greater decrease recorded in IH (down 8.8 ± 3.2%) and PH (down 9.4 ± 3.3%) conditions relative to EH (down 4.2 ± 2.0%; $p < 0.05$ vs IH and PH). Figure 3 shows these cerebral haemodynamic changes for each experimental condition relative to the proceeding IE baseline.

Cognitive Task Performance

Simple and Complex Reaction Time: Performance scores for both reaction time tasks are shown in Table 2. Baseline IE measures were consistent between all conditions ($p > 0.05$). During IH, performance times for SRT (566 ± 50 ms) and CRT (594 ± 70 ms) tasks were unaffected with respect to IE ($p > 0.05$), whereas PH caused a significant slowing of both

SRT (by 149 ± 81 ms; $p < 0.001$ vs IH) and CRT (by 152 ± 82 ms; $p < 0.001$ vs IH) performance. For the subgroup, EH produced similar performance decrements as was observed during PH ($p > 0.05$) for both SRT (slower by 174 ± 42 ms; $p < 0.001$ vs IH) and CRT (slower by 167 ± 70 ms; $p < 0.001$ vs IH) performance. There was no effect of condition on SRT and CRT error count.

Spatial Working Memory Task: Error count for the SWM task is shown in Table 2. There was no significant change in error count during the experimental conditions compared to IE conditions for any protocols.

Relation between cerebral haemodynamics and cognitive task performance

Finally, as shown in figure 4A and B, changes in MCAv were moderately correlated ($R^2 = \sim 0.28$) with both SRT and CRT, such that increases in MCAv were associated with maintained reaction time task performance. These correlations were not apparent in the NIRS-derived prefrontal cortex measures of tissue saturation and perfusion (as indexed by TOI and nTHI, respectively), with no significant correlations observed (all $p > 0.05$, see Figures 4C-F).

Discussion

The present study was designed to investigate the independent roles of hypoxia and hypocapnia on simple and complex cognitive ability, and how changes in global and prefrontal cerebral haemodynamics were associated with altered cognitive performance. We found that acute exposure to PH impaired both SRT and CRT performance, but it had no apparent effect on SWM task performance. Hypocapnia alone (i.e. EH) produced similar decrements to those seen during PH, whilst the supplementation of carbon dioxide to maintain $P_{ET}CO_2$ relieved the hypoxia-induced cognitive impairment. The associated changes in cerebral haemodynamics indicate that differences in CBF between the experimental conditions may mediate this effect, with the changes in global flow (as indexed by MCAv) moderately correlated to cognitive task performance. Interestingly, despite differences in global flow and the associated link to performance, prefrontal cerebral tissue perfusion and saturation were not different between hypoxic trials and not linked to cognitive performance. Overall, these findings reveal a significant role of hypocapnia *per se* on the development of cognitive impairment during normoxic *and* hypoxic exposures.

Cognitive Function during Hypoxia and Hypocapnia

The observed detriment to cognitive function during PH reported in the current study is consistent with previous work showing impairment in CRT during exposure to high altitude (Dykiert et al., 2010). Further, our findings of the recovered cognitive performance during carbon dioxide supplementation in hypoxia has also been previously demonstrated (Van Dorp et al., 2007). However, to our knowledge no such cognitive impairment has been found when tasks are completed under hypocapnia when controlling for hypoxia, nor demonstrated how cerebral haemodynamic changes may mediate this effect (discussed below). Interestingly, Bloch-Salisbury and colleagues reported significant changes to electroencephalographic signals under hypocapnia during a series of rapid-response cognitive tasks (Bloch-Salisbury, Lansing, & Shea, 2000); however, these changes did not reflect impairment to response time or error scores despite a similar hypocapnic stimulus ($P_{ET}CO_2$ of ~30mmHg) to that induced in the current study. The present data exhibited a speed-accuracy trade-off for SRT and CRT performance during PH and EH conditions, with significantly slower performance times recorded with no change to error count. An unexpected finding of the present study was that the performance of the SWM task was unaffected by all conditions. It is widely accepted that as altitude increases, complex cognitive abilities, such as working memory become progressively impaired (reviewed in Yan, 2014). Studies using test batteries to examine executive function performance during hypoxia have found impairments in the Paced Auditory Serial Addition Test (PASAT) (Malle et al., 2013) and Stroop Test (Turner, Barker-

Collo, Connell, & Gant, 2015) task performance. Despite differences in mean average error count, it is likely that we did not have the power (effect size = 0.279, observed power = 0.498) to detect any significant differences in SWM task performance as a consequence a lack of sensitivity of the SWM CANTAB task. Further, Lowe and Rabbitt (1998) described that for executive function to be measured effectively tasks must remain novel to the participant due to the rapid improvements in performance once an optimal strategy is discovered. Specifically, the familiarisation session conducted to minimise the learning effects may have provided a ceiling effect for SWM task performance The CANTAB SWM task used here is designed to test memory retention, strategy, and visuospatial abilities as a representation of executive function. The version of the SWM task used in this present study produces 15 identical arrangements of coloured boxes for each repeat, which may diminish its ability to reliably measure executive function. Patients with mild cognitive impairment and Alzheimer's disease completing the CANTAB SWM task in a 6 month test-retest assessment are shown to exhibit a practice effect by optimising their strategy search patterns, which was maintained at the 12-month re-test assessment (Cacciamani et al., 2018). Subsequently in the present study, the acute test-retest period that was used (within ~1 hr) would likely have been compromised by this learning effect. In addition, the measurements of error collected by the SWM task may not provide adequate information to determine whether there is impairment to performance. Based on our reaction time task performance decrements, it was the speed of the response that was impaired as opposed to the accuracy. As such, including a time pressure during a cognitive task may be a more effective way to demonstrate the hypoxic impairment effect given its effect on a recall task (Earles, Kersten, Berlin Mas, & Miccio, 2004). Indeed, this is consistent with observations of hypoxia-related impairment of PASAT test performance (Malle et al., 2013), a task which includes a time pressure.

Cerebral Haemodynamics and Cognitive Function

Exposure to hypoxia is well known to cause a cerebral vasodilatory response but is compromised by the reflex hypoxia-induced hyperventilation response lowering $PaCO_2$ and causing cerebral vasoconstriction (Ainslie & Ogoh, 2010). In the present study, there was no change in MCAv observed during PH, reflecting the contrasting cerebrovascular activity that hypoxia and hypocapnia stimulate (Mardimae et al., 2012). Consistent with previous observations, the supplementation of carbon dioxide to maintain $P_{ET}CO_2$ constant during the hypoxic exposure (i.e. IH) allowed the cerebrovasculature to dilate and thus to increase oxygen delivery to the brain via elevated flow (Van Dorp et al., 2007). Indeed, higher blood flow velocity was associated with maintained reaction time task performance (Fig 4A and 4B). Interestingly, while increases in global cerebral haemodynamics were observed during IH compared to PH (and EH), the NIRS-based measures of regional tissue perfusion as indexed by haemoglobin content (i.e. nTHI) at the prefrontal cortex was not different between the hypoxia conditions, which increased similarly in both hypoxic conditions. A potential explanation is that this may reflect a global increase in CBF during IH, compared to a regional shift of blood towards active areas of the brain during PH, particularly at the prefrontal cortex. Binks and colleagues reported a global increase in CBF to all areas of the brain during IH, but not necessarily each to the same magnitude (Binks, Cunningham, Adams, & Banzett, 2008). Additionally, Lawley et al. reported an active heterogeneous CBF response following two hours of PH, with increased perfusion observed in the anterior portions of the brain and reductions to the posterior regions (Lawley, Macdonald, Oliver, & Mullins, 2017). It is known that different portions of the brain are activated depending on the task completed, with working memory processes stimulating the prefrontal cortex (van Asselen et al., 2006), whereas reaction time tasks activate both the premotor and primary sensorimotor areas (Kwon, Kwon, & Park, 2013). This regional activation may explain why no significant haemodynamic differences were seen between the impaired reaction time tasks and the unimpaired SWM task as only prefrontal cortex measurements were recorded. Further investigation using whole-head functional imaging would enable a clearer understanding of the regional differences in CBF during cognitive tasks under hypoxia and hypocapnia.

Despite possible differences in the maintenance of local blood flow, there was an equivalent fall in cerebral oxygenation (TOI) observed in both IH and PH, indicating that insufficient delivery of oxygen to the tissue is not the defining factor behind the cognitive function difference. This is demonstrated with no meaningful correlations found between TOI and reaction time task performance (Fig 4C-D). Hypocapnia causes haemoglobin to have an increased affinity for oxygen and reduce oxygen unloading at the tissues (Collins, Rudenski, Gibson, Howard, & O'Driscoll, 2015). This may be a defining factor between the two hypoxic conditions in the development of cognitive impairment, with the supplementation of carbon dioxide reversing the leftward shift of the oxygen-haemoglobin dissociation curve, allowing adequate offloading of oxygen into the tissue. This is highlighted during EH given that there was less of a fall in TOI but still a cognitive impairment. With hypoxia-induced hypocapnia comes respiratory alkalosis and acid-base adjustment via renal compensation through excretion of bicarbonate ion (HCO_3^-), although this is typically reported with longer exposures than the 60 minutes we used here. Further, it remains undefined whether $PaCO_2$ or pH acts as the primary stimulant responsible for cerebral vasoconstriction (Willie, Tzeng, Fisher, & Ainslie, 2014). Nonetheless, hypocapnia-induced vasoconstriction has been shown to impact the neurovascular coupling response, such that it overwhelms the neuronal activated vasodilation response to visual stimulation, and compromises oxygen supply to the brain (Szabo et al., 2011). The combination of a compromised oxygen supply and reduced oxygen unloading causes hypocapnia-induced brain ischaemia (Laffey & Kavanagh, 2002) and could well be an underlying factor in the development of the cognitive impairment during hypoxic exposure. In addition to altering the neurovascular coupling response, the cerebral metabolic rate of oxygen ($CMRO_2$) does not change during isocapnic hypoxia (Ainslie et al., 2014), with MRI-based evidence indicating that increased neural excitability (and subsequent $CMRO_2$) during hypoxia are as a consequence of hypoxic ventilatory response-induced hypocapnia (Smith et al., 2012; Vestergaard et al., 2015). This increase in $CMRO_2$ has been shown to be mitigated during hypoxia with the administration of acetazolamide (Wang, Smith, Buxton, Swenson, & Dubowitz, 2015), which indicates an important role of hypocapnia and alkalosis in cerebral metabolism during acute hypoxia.

In the present study, the use of an acute exposure to normobaric hypoxia enables the controlled manipulation of oxygen and carbon dioxide to investigate their impact on cognitive function. During extended or chronic exposures to hypobaric hypoxia (i.e. the natural high-altitude environment), a complex integrative response to hypoxia will also include haematological and extended nephrological compensation in addition to regulation by arterial blood gases. Consequently, the effect of respiratory alkalosis on CBF, metabolism and cognitive function is likely to be influenced by the degree of hypoxic ventilatory response and renal compensation during acclimatization. Similarly, haemoglobin increases occur within weeks of high altitude exposure and improve CaO_2 and global oxygen delivery (Subudhi et al., 2014). Therefore, cognitive impairment to tasks involving sustained attention (i.e. tasks involving reaction time) often occur during the initial exposure to high altitude (4,350m and 5,050m), but are reversed within the days following acclimatization (Davranche et al., 2016; Pun et al., 2018).

Methodological Considerations

An important consideration to acknowledge is that during EH $P_{ET}CO_2$ was not matched to the changes in $P_{ET}CO_2$ induced during PH (i.e. $P_{ET}CO_2$ significantly different between PH and EH conditions). Our aim was to elicit a hypocapnic state that resembled the one that results from the natural hyperventilation caused by hypoxia, but in reality we overestimated this response when selecting the target $P_{ET}CO_2$ in EH. This could have been avoided if all participants had undertaken the EH condition after the PH condition, but of course this would then introduce a problematic order effect. Nevertheless, studies report that there is a linear graded response of cerebral saturation with carbon dioxide tensions (Mutch et al., 2013), and so mechanisms by which hypocapnia induces cognitive impairment may also work in a graded fashion.

Active hyperventilation is attention consuming when compared to passive hyperventilation (Gallego, Perruchet, & Camus, 1991), and subsequently may confound any interpretation of hypocapnia on cognitive functioning. To overcome this, previous studies have assessed cognitive function during the minutes of recovery from hyperventilation-induced hypocapnia (Van Diest et al., 2000). However, our battery of cognitive tasks took approximately 15 minutes to complete, which was too long to use such an approach. Indeed, Malatino and colleagues demonstrated that MCAv returns to near baseline values within five minutes following hyperventilation-induced hypocapnia, and this was from a greater level of hypocapnia ($P_{ET}CO_2$=20 mm Hg) than induced in the current study (Malatino et al., 1992). Nonetheless, completing a normocapnic normoxic hyperventilation trial would determine the effect of active hyperventilation on the cognitive function. Transcranial Doppler measures blood flow velocity as an index of vessel blood flow based on the assumption that the diameter of the MCA remains constant. This assumption has recently been questioned (Ainslie & Hoiland, 2014) and evidence for altered MCA diameter in conditions where blood gas content is affected has been demonstrated (Coverdale, Gati, Opalevych, Perrotta, &

Shoemaker, 2014; Verbree et al., 2014; Wilson et al., 2011). Nevertheless, if the diameter of the MCA was increased (in IH) or decreased (EH) as a consequence of the manipulated blood partial pressures, our TCD-based findings would only underestimate the true effect observed here.

As mentioned above, we only measured prefrontal cerebral haemodynamic changes with our NIRS and so the regional perfusion shifts proposed would need to be confirmed via whole-head NIRS imaging (or with functional magnetic resonance imaging). Further, NIRS is limited to the cortex surface and currently available technology and analysis approaches does not differentiate between skin and skull blood flow, and cerebrospinal fluid. However, despite its spatial limitations NIRS is clearly able to measure changes in haemodynamic responses, and which are more likely to result from neural activation than haemoglobin content shift within the blood vessels of the skin under this experimental paradigm (Davies et al., 2016; Davies et al., 2017). Finally, these apparatuses only reflect global CBF and regional haemoglobin content, representing vascular flow and oxygenation changes to our measured areas of interest. Neither of these imaging devices provided any measure of cerebral metabolic rate of oxygen, which may better reflect the mechanisms of cognitive (dys)function during hypoxia and hypocapnia exposure and warrants future study.

Conclusion

Hyperventilation-induced hypocapnia impairs performance of simple and five-choice reaction time tasks during normoxia and hypoxia, but not working memory cognitive performance. Furthermore, supplementation of carbon dioxide during hypoxia preserved cognitive function and facilitates an appropriate vascular response. The associated changes in global cerebral haemodynamics between the experimental conditions may mediate this effect, with the changes in MCAv moderately correlated to cognitive task performance. Taken together, these findings reveal the significant role of hypocapnia *per se* on the development of cognitive impairment during normoxic *and* hypoxic exposures.

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Absolute Resting Haemodynamic and Gas Values during Isocapnic Euoxic and Experimental Conditions

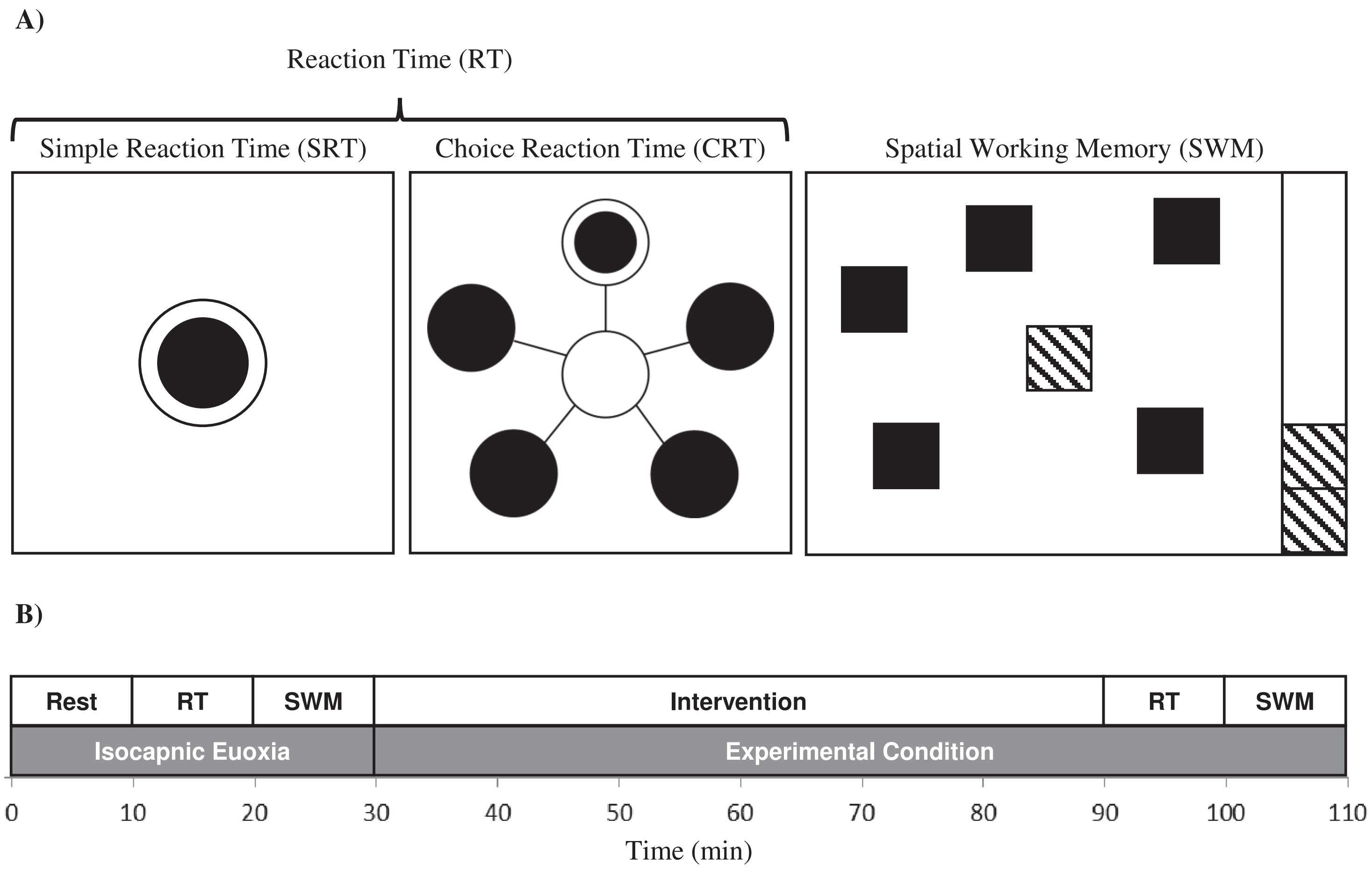
	HR (bpm)	MAP (mmHg)	MCAv (cm·s^{-1})	TOI (%)	nTHI (au)	$P_{ET}O_2$ (mmHg)	$P_{ET}CO_2$ (mmHg)
Isocapnic Euoxic (n=20)							
Isocapnic Hypoxia	67.3 ± 10.8	82.4 ± 15.7	65.4 ± 11.8			98.9 ± 3.9	41.1 ± 2.1
Poikilocapnic Hypoxia	68.1 ± 11.3	87.6 ± 14.7	64.9 ± 12.2			98.8 ± 4.2	41.5 ± 2.0
Subgroup (n=10)							
Isocapnic Hypoxia	60.9 ± 7.2	77.4 ± 10.9	61.6 ± 12.2	74.9 ± 4.9	0.98 ± 0.08	99.6 ± 4.0	40.8 ± 1.7
Poikilocapnic Hypoxia	63.4 ± 8.2	82.3 ± 11.8	60.9 ± 11.0	73.7 ± 3.7	1.00 ± 0.06	100.8 ± 3.8	41.2 ± 2.4
Euoxic Hypocapnia	63.4 ± 8.7	77.4 ± 12.7	61.9 ± 9.9	73.3 ± 3.8	1.00 ± 0.05	97.0 ± 3.3	41.6 ± 1.3
Experimental (n=20)							
Isocapnic Hypoxia	68.9 ± 10.3	90.8 ± 14.0	72.2 ± $11.8^{\alpha**}$			43.6 ± 1.7^{**}	41.4 ± 2.2^{α}
Poikilocapnic Hypoxia	71.0 ± 10.6	86.4 ± 10.3	67.3 ± 11.0			42.2 ± 2.9^{**}	39.0 ± 3.2^{**}
Subgroup (n=10)							
Isocapnic Hypoxia	63.9 ± 6.3	87.5 ± 14.4	68.5 ± $10.8^{\alpha\beta*}$	66.3 ± $4.6^{\beta**}$	1.02 ± $0.09^{\beta*}$	43.4 ± $1.8^{\beta**}$	41.3 ± $2.1^{\alpha\beta}$
Poikilocapnic Hypoxia	65.1 ± 8.6	84.2 ± 9.9	62.2 ± 9.1	64.7 ± $4.8^{\beta**}$	1.03 ± 0.08^{β}	42.5 ± $3.6^{\beta**}$	37.9 ± $3.5^{\beta**}$
Euoxic Hypocapnia	61.0 ± 9.4	85.6 ± 11.3	51.7 ± 7.6^{**}	68.8 ± 4.1^{**}	0.95 ± 0.05^{**}	97.0 ± 3.6	33.3 ± 1.5^{**}

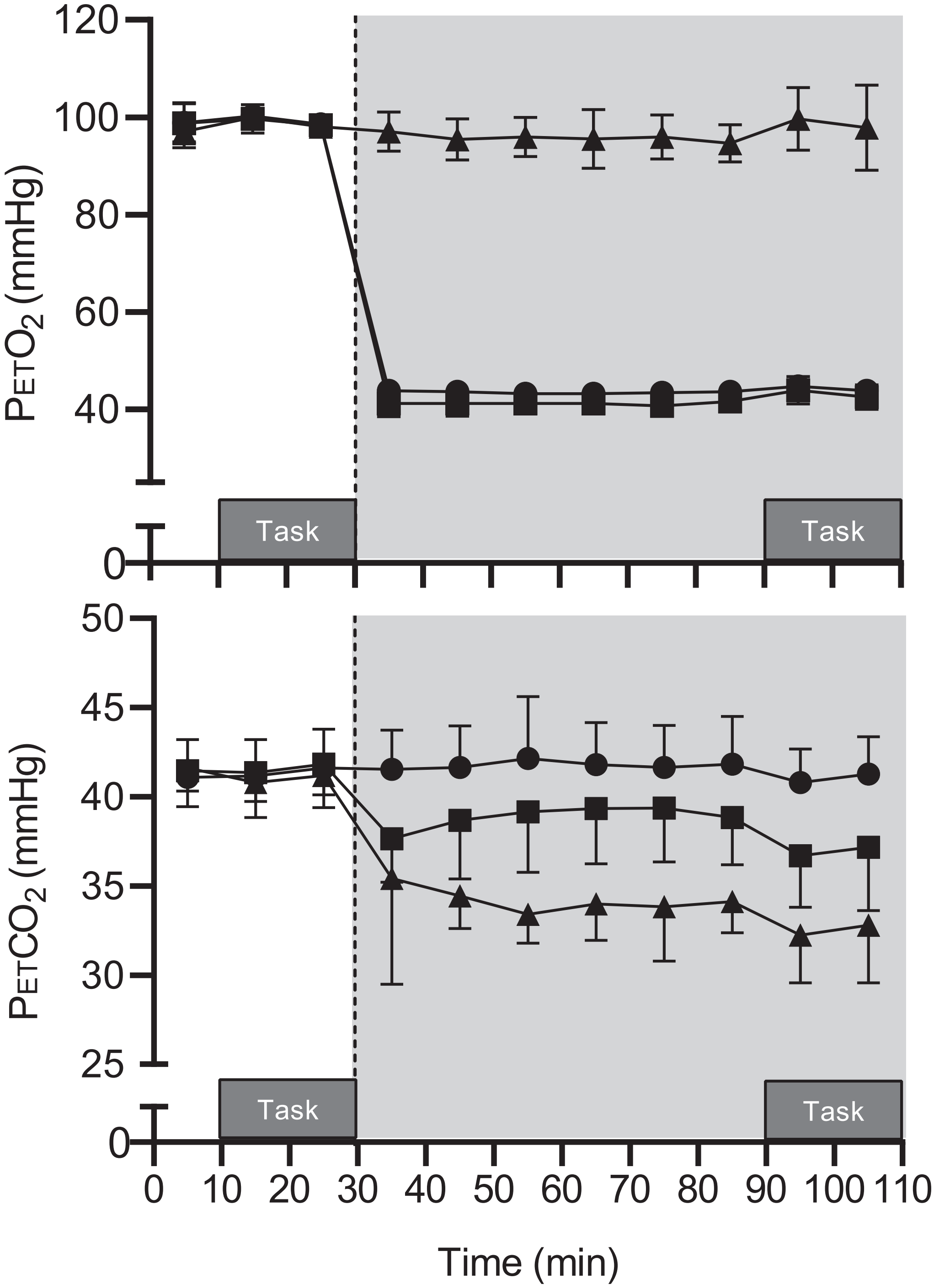
Table 1. Absolute resting values for cerebral haemodynamics and end-tidal respiratory gases during isocapnic euoxic baseline and experimental conditions. Experimental conditions were isocapnic hypoxia (IH), poikilocapnic hypoxia (PH), and euoxic hypocapnia (EH). Data are presented for the group which completed IH and PH conditions (n = 20), and for the subgroup which completed the additional EH condition (n = 10). Significance notation represents differences between data pooled across four measured time points during each IE and experimental period. * $p < 0.05$ compared to IE. ** $p < 0.001$ compared to IE. α $p < 0.05$ compared to PH. β $p < 0.05$ compared to EH. HR, Heart rate; MAP, Mean arterial pressure; MCAv, Middle cerebral artery velocity; TOI, Total oxygenation index; nTHI, Total haemoglobin index normalised to initial value; $P_{ET}O_2$, End-tidal partial pressure of oxygen; $P_{ET}CO_2$, End-tidal partial pressure of carbon dioxide Values are Mean ± SD.

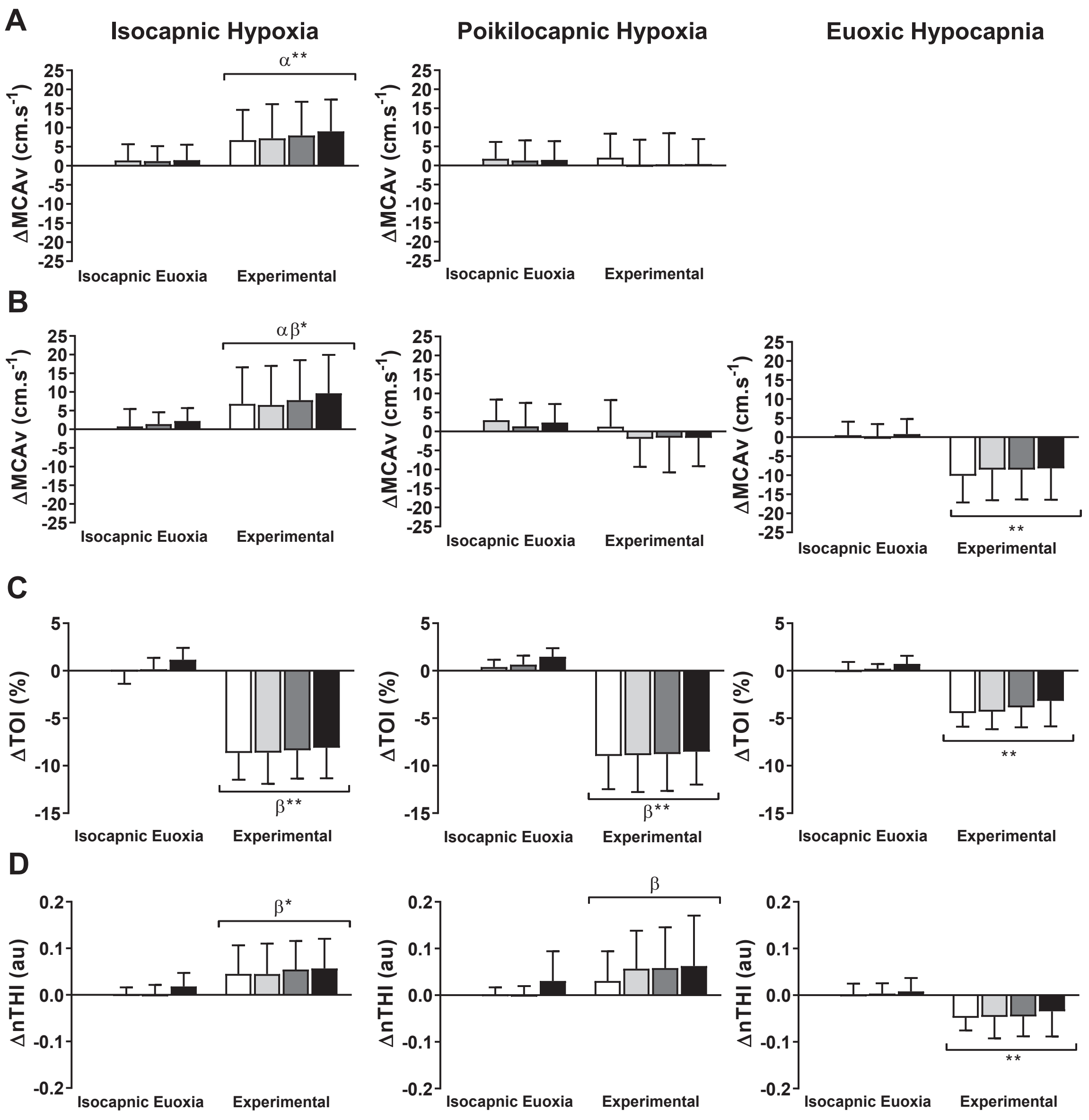
Cognitive Task Performance during Isocapnic Euoxic and Experimental Conditions

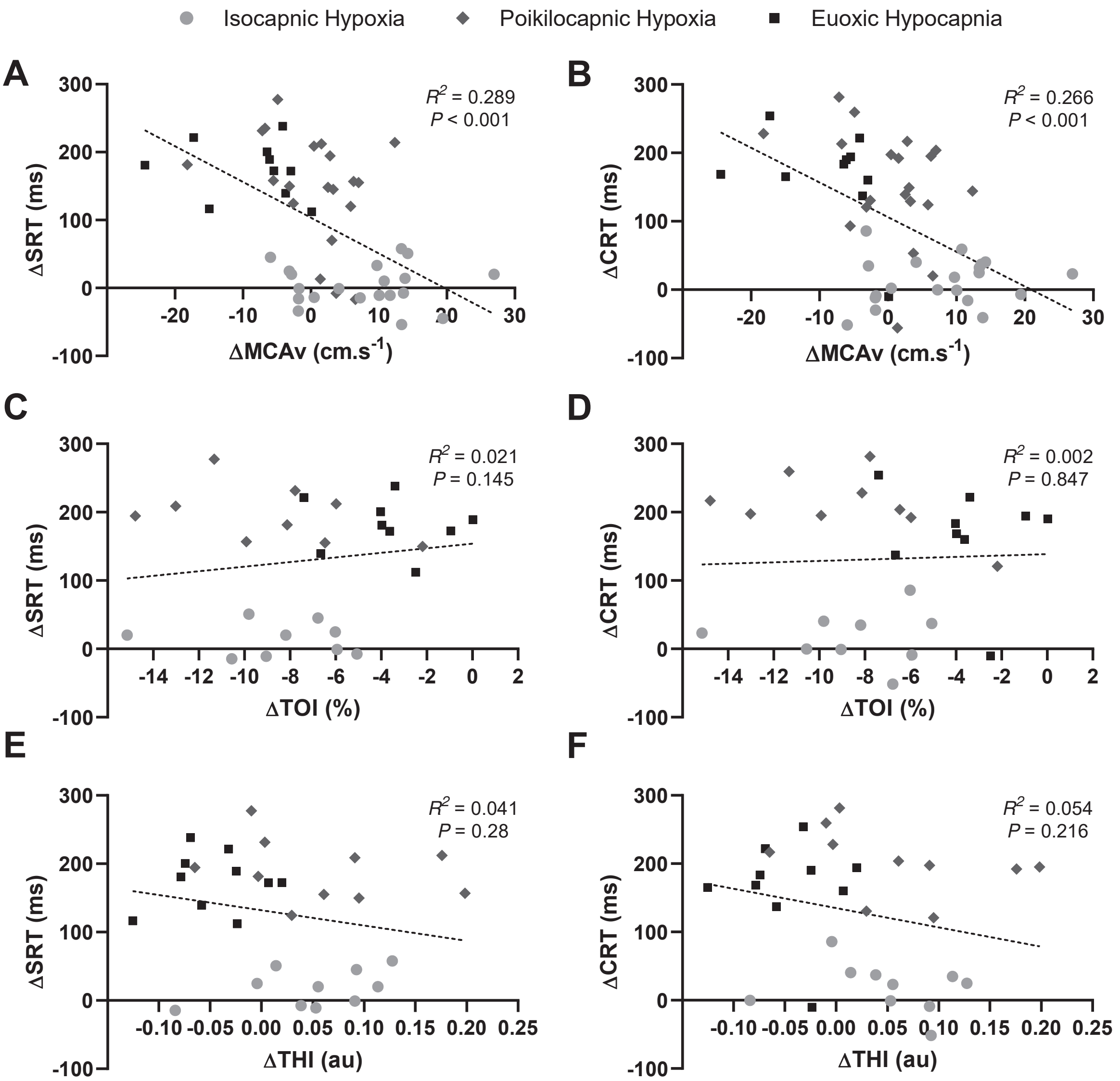
	SRT		CRT		SWM
	Time (ms)	Error Count	Time (ms)	Error Count	Error Count
Isocapnic Euoxic (n=20)					
Isocapnic Hypoxia	573 ± 55	0.3 ± 0.5	590 ± 60	0.8 ± 0.7	7.9 ± 12.8
Poikilocapnic Hypoxia	552 ± 52	0.4 ± 0.5	583 ± 74	0.9 ± 1.3	9.5 ± 12.9
Subgroup (n=10)					
Isocapnic Hypoxia	550 ± 47	0.3 ± 0.5	579 ± 58	0.8 ± 0.6	8.1 ± 15.9
Poikilocapnic Hypoxia	532 ± 46	0.6 ± 0.5	562 ± 72	1.4 ± 1.5	7.3 ± 15.1
Euoxic Hypocapnia	544 ± 31	0.5 ± 0.7	588 ± 69	0.6 ± 0.8	5.6 ± 11.8
Experimental (n=20)					
Isocapnic Hypoxia	575 ± 54	0.5 ± 0.6	600 ± 75	0.8 ± 0.8	8.1 ± 17.5
Poikilocapnic Hypoxia	700 ± 85$^{\delta**}$	0.3 ± 0.4	735 ± 86$^{\delta**}$	0.6 ± 0.8	11.5 ± 13.3
Subgroup (n=10)					
Isocapnic Hypoxia	566 ± 51	0.3 ± 0.5	594 ± 70	1.0 ± 0.8	6.4 ± 14.0
Poikilocapnic Hypoxia	721 ± 51$^{\delta**}$	0.3 ± 0.5	765 ± 47$^{\delta**}$	0.6 ± 1.0	9.8 ± 17.2
Euoxic Hypocapnia	718 ± 55$^{\delta**}$	0.6 ± 0.8	755 ± 34$^{\delta**}$	0.2 ± 0.6	13.0 ± 15.5

Table 2. Performance time and error count for simple reaction time (SRT) and five-choice reaction time (CRT) tasks, and error count for spatial working memory (SWM) task during isocapnic euoxic and experimental conditions. Experimental conditions were isocapnic hypoxia (IH), poikilocapnic hypoxia (PH) and euoxic hypocapnia (EH). Data have been presented for the group (n=20) which completed the IH and PH conditions, and for the subgroup (n=10) which completed the additional EH condition. ** $p < 0.001$ compared to IE. δ $p < 0.001$ compared to IH. Values are Mean ± SD


A)
Reaction Time (RT)
Simple Reaction Time (SRT)
Choice Reaction Time (CRT)
Spatial Working Memory (SWM)
B)
Rest
RT
SWM
Intervention
RT
SWM
Isocapnic Euoxia
Experimental Condition
0
10
20
30
40
50
60
70
80
90
100
110
Time (min)


120
100
80
60
40
0
$P_{ET}O_2$ (mmHg)
Task
Task
50
45
40
35
30
25
0
$P_{ET}CO_2$ (mmHg)
Task
Task
0
10
20
30
40
50
60
70
80
90
100
110
Time (min)


A
Isocapnic Hypoxia
Poikilocapnic Hypoxia
Euoxic Hypocapnia
ΔMCAv (cm.s-1)
25
20
15
10
5
0
-5
-10
-15
-20
-25
α**
Isocapnic Euoxia
Experimental
B
αβ*
**
C
ΔTOI (%)
5
0
-5
-10
-15
β**
D
ΔnTHI (au)
0.2
0.1
0.0
-0.1
-0.2
β*
β


● Isocapnic Hypoxia
◆ Poikilocapnic Hypoxia
■ Euoxic Hypocapnia
A
ΔSRT (ms)
ΔMCAv (cm.s-1)
R^2 = 0.289
P < 0.001
B
ΔCRT (ms)
ΔMCAv (cm.s-1)
R^2 = 0.266
P < 0.001
C
ΔSRT (ms)
ΔTOI (%)
R^2 = 0.021
P = 0.145
D
ΔCRT (ms)
ΔTOI (%)
R^2 = 0.002
P = 0.847
E
ΔSRT (ms)
ΔTHI (au)
R^2 = 0.041
P = 0.28
F
ΔCRT (ms)
ΔTHI (au)
R^2 = 0.054
P = 0.216
300
200
100
0
-100
-20
-10
0
10
20
30
-14
-12
-10
-8
-6
-4
-2
0
2
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
0.25